

Highly Purified *Pseudomonas aeruginosa* LPS

Product	Cat. #	Price/100ug
<i>P. aeruginosa</i>	7000	\$95

INTRODUCTION

Pseudomonas aeruginosa (*PSa*) is a gram negative bacteria responsible for chronic lung infections in patients with cystic fibrosis. Like all gram negative bacteria, *PSa*, generates lipopolysaccharide (LPS), a cell wall component, which is a key modulator of the host's innate defense response to bacterial infection. *PSa*, when purified by phenol, produces heterogeneous species of lipid A, the active portion of the LPS molecule. This fraction of *PSa* LPS lipid A contains mostly molecular negative mass ions of 1447 and 1616, representing penta-acylated and hexa-acylated lipid A, respectively. This specific fraction is a weak agonist for the following inflammatory mediators: E-Selectin expression by HUVEC and IL-8 expression by macrophages.

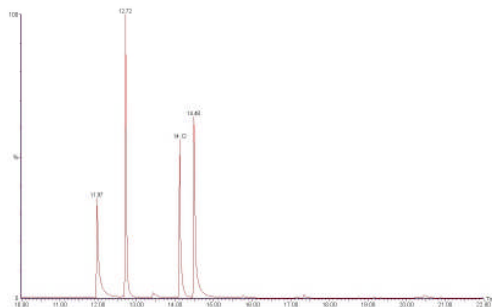
BIOCHEMICAL ANALYSIS

Purity

	<i>PSa</i>
DNA content	1%
Protein content	0.43%

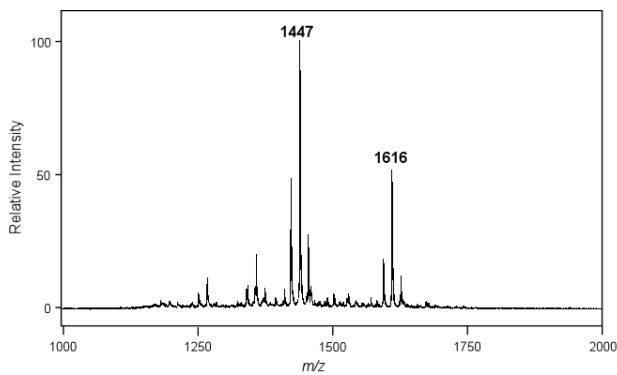
Gas chromatographic/mass spectroscopy (GC/MS) of LPS fatty acids.

PSa LPS samples were analyzed by their trimethylsilyl ethers after transmethylation with N,O-bis(trimethylsilyl) trifluoroacetimide (BSTFA) containing 1% trimethylchlorosilane (TMCS). The fatty acids are accounted for and no other fatty acids nor phospholipid, glycolipid, or lipoprotein were detected.

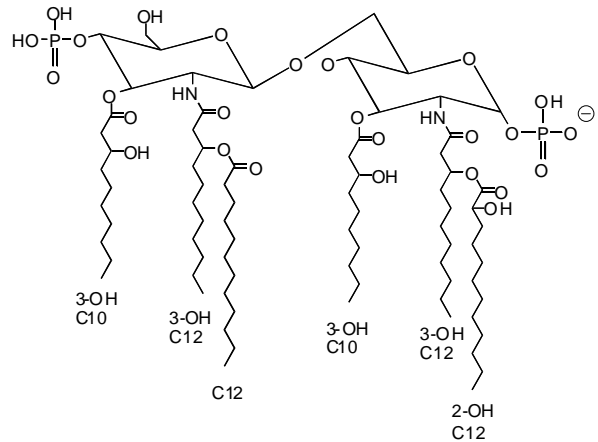


Mass Spectrometry

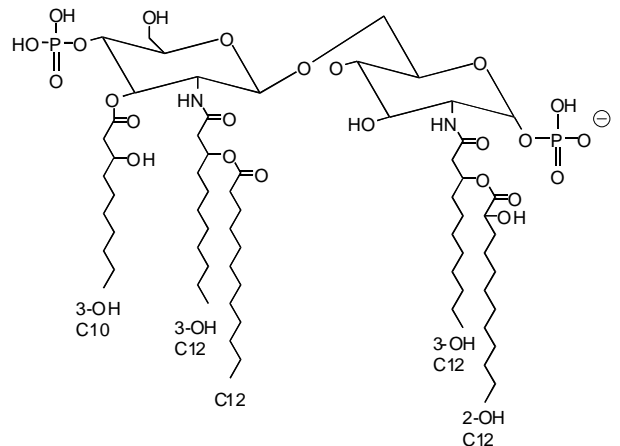
The deduced structure of the Lipid A moiety of *PSa* was analyzed by matrix assisted laser desorption ionization time of flight (MALDI-TOF). This fraction of *PSa* LPS lipid A contains mostly molecular negative mass ions of 1447 and 1616, representing penta-acylated and hexa-acylated lipid A, respectively



m/z 1616

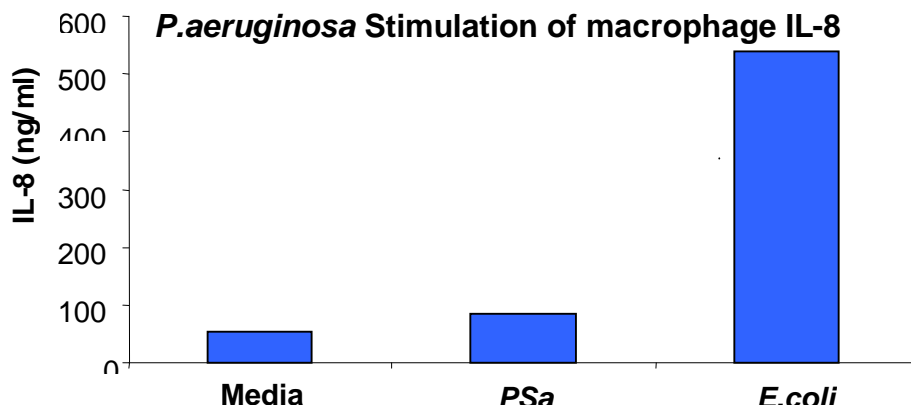
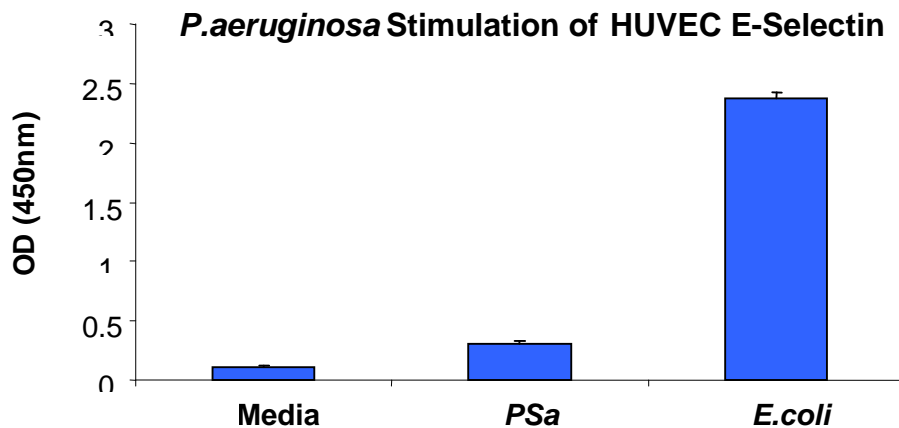


m/z 1447



BIOLOGIC ACTIVITY

The biologic response of the purified LPS was tested for E-Selectin expression on HUVEC cells (Astarte HUVEC/E-Selectin Kit #2016). These cells normally produce E-selectin in response to *E.coli* or its purified LPS. The purified *PSa* LPS (1.0 $\mu\text{g/ml}$) only weakly stimulated E-selectin expression compared to *E.coli* (10ng/ml). The purified *PSa* LPS (1.0 $\mu\text{g/ml}$) also only weakly stimulated macrophages to secrete IL-8, compared to *E.coli* (1.0 $\mu\text{g/ml}$).



REFERENCES

1. Ernst RK, Yi EC, Guo L, Kheng BL, Burns JL, Hackett M, and Miller SI. Specific Lipopolysaccharide Found in Cystic Fibrosis Airway *Pseudomonas aeruginosa*. *Science* 1999;286:1561-1565.
2. Darveau RP, Cunningham MD, Bailey T, et al. Ability of bacteria associated with chronic inflammatory disease to stimulate E-selectin expression and promote neutrophil adhesion. *Infect Immun* 1995;63:1311-1317.